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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

40399/299/NIHD

In re patent application of
Jeffrey RUBIN *et al.*
Serial No. 08/455,975
Filed: May 31, 1995
For: DNA ENCODING A GROWTH FACTOR SPECIFIC FOR EPITHELIAL CELLS

Group Art Unit: 1812
Examiner: Saoud

Saoud

LETTER

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Pursuant to a telephone conference with Examiner Saoud on January 14, 1997, attached is Page 40 of the Substitute Specification which was inadvertently omitted on December 10, 1996. We take this opportunity to thank Examiner Saoud for her attention to this matter and for the telephone call made to the undersigned.

Respectfully submitted,

Date: January 14, 1997

Patricia D. Granados
Reg. No. 33,683

FOLEY & LARDNER
Suite 500
3000 K Street, N.W.
Washington, DC 20007-5109
(202) 672-5300

as well as the naturally occurring KGF in a solid-phase (ELISA) assay. None cross-reacts with KGF under denaturing conditions (in a Western blot), and none neutralizes mitogenic activity of KGF in the BALB/MK bioassay.

Polyclonal antibodies were generated with a synthetic peptide with the amino acid sequence NDMTPEQMATNVR, corresponding to residues numbered 32 through 44 in KGF (see Fig. II-1), plus an R (Arg) residue instead of the actual Asn residue encoded by the cDNA at position 45. The Asn residue is probably glycosylated in the natural KGF polypeptide and, therefore, was not identified in the amino acid sequencing data obtained directly from that polypeptide (see Discussion, below). Polyclonal antibodies generated with this synthetic peptide recognize both naturally occurring and recombinant KGF in ELISA and Western blot analyses at a level of sensitivity of at least as low as 10 ng protein. These antibodies, however, do not neutralize mitogenic activity of KGF in the BALB/MK bioassay.

Polyclonal antisera against intact natural KGF protein recognizes KGF in both ELISA and Western blot assays. Such antibodies also appear to inhibit mitogenic activity of KGF in the BALB/MK bioassay.

Expression of KGF cDNA in *E. coli*. KGF cDNA was expressed to produce polypeptide, in *E. coli* by placing its coding sequence under control of the hybrid trk promoter (comprising elements of trp and lac promoters), in the plasmid pKK233-2 (Amman, E. and Brosius, J. (1985) Gene 40, 183). To accomplish this, a specific length of KGF cDNA that contained the information to code for the mature KGF molecule (i.e., without its signal peptide) was amplified using the polymerase chain reaction technique (Sakai, R.K., Scharf, S., Faloona, F., Mullis, K.B., Norn, G.T., Erlich, H.A. and Arnheim, N. (1985) Science 230, 1350-1354). The fragment was directionally inserted between two sites in the vector, namely the *Nco*I site, made blunt ended by S1 nuclease digestion, and the *Hind*III site, using standard recombinant DNA methodology.